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The hypercholesterolemic effect of cafestol in coffee oil in gerbils and rats

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Coffee beans contain the diterpene cafestol, which raises plasma cholesterol concentrations in humans. Daily consumption of 2 g coffee oil, which provides approximately 60 mg cafestol (equivalent to 5.7 mg cafestol/MJ), increases plasma cholesterol concentrations by 28%. We studied the effect of cafestol in coffee oil on gerbils and rats to determine whether the pathways that lead to cafestol-induced hypercholesterolemia in humans are also present in other species. We fed coffee oil from the same batch used in humans to female gerbils and rats. Gerbils were fed a semipurified diet containing 0.5% or 5% (w/w) coffee oil (equivalent to 8.7 and 86.8 mg cafestol/MJ, respectively) in the presence or absence of 0.05% (w/w) cholesterol for a period of 10 weeks. When compared with the gerbils fed no coffee oil, the addition of 0.5% coffee oil to the diets did not affect plasma cholesterol. Plasma cholesterol was significantly higher only when 5% coffee oil was fed, both in the absence (1.01 mmol/L, 33% higher) and presence (1.87 mmol/L, 70% higher) of dietary cholesterol. Liver weight was also significantly higher when 5% coffee oil was fed. Rats were also fed diets containing 0.5% or 5% coffee oil (equivalent to 8.7 and 86.8 mg cafestol/MJ) with and without 0.05% cholesterol for 8 weeks. Feeding 0.5% coffee oil compared with no coffee oil resulted in significantly higher plasma cholesterol levels throughout the study both in the absence (0.46 mmol/L, 27% higher) and presence (0.28 mmol/L, 15% higher) of dietary cholesterol. Diets containing 5% coffee oil appeared to be toxic. Thus, coffee oil diterpenes can result in higher plasma cholesterol in gerbils and rats. The failure to observe these effects in previous studies may be due to doses that were too low. (J. Nutr. Biochem. 11:311–317, 2000)

Keywords: cafestol; coffee oil; cholesterol; gerbils; rats

Introduction

Epidemiologic studies in Scandinavian countries showed a positive correlation between coffee intake and plasma cholesterol concentrations.[1] Such a relationship has not been consistently found in other Western European countries and the United States.[2] Controlled studies indicated that these different findings could be explained by the method of brewing the coffee. Unfiltered, boiled coffee raised plasma cholesterol whereas filtering the coffee eliminated this effect.[3,4] Further studies indicated that boiled, unfiltered coffee contains a small amount of lipid that raises plasma cholesterol and is retained by the filter. This coffee lipid contains two types of diterpenes, cafestol and kahweol, which are responsible for the cholesterol-raising properties of unfiltered coffee.[5,6] The cholesterol-raising effect is mainly attributable to cafestol.[7]

The effect of coffee diterpenes in animals is of interest for two reasons: (1) to provide insight into the comparative physiology of laboratory animals and humans and (2) to provide a model in which the mechanism of the cholesterol-raising action of diterpenes can be studied. Unfiltered coffee and cafestol have consistently raised cholesterol in humans, but the effect is less clear in experimental animals. Sanders and Sandadura[8] and Ratnayake et al.[9] reported that cholesterol levels in hamsters responded to boiled coffee and coffee oil, but other studies in this animal species were unsuccessful.[10,11] Boiled coffee resulted in higher cholesterol levels in one study with rats,[12] but in other studies with rats no effect of boiled coffee was seen.[10,13] Similarly,

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studies with gerbils[11] did not show a cholesterolemic effect of boiled coffee. In both Rhesus and Cebus monkeys the feeding of diets containing coffee oil did not influence serum cholesterol concentrations.[14]

In the present study we examined the effect of cafestol present in coffee oil on plasma cholesterol in gerbils and rats. We used the same batch of coffee oil as was used in earlier studies with humans.[6] We fed proportions of coffee oil and cafestol (expressed in grams per megajoule) similar to those used in the studies with humans. It is possible that the cholesterolemic effect of cafestol in coffee oil in experimental animals becomes evident only at a dose higher than that fed to humans. Therefore, we also fed groups of animals with higher proportions of coffee oil and cafestol. Part of the results of this study have been published in abstract form.[15]

Methods and materials

The protocols of the experiments were approved by the animal experiments committee of the Wageningen Agricultural University.

Animals and diets

Ninety 4-week-old female gerbils (*Meriones unguiculatus*) were purchased from Janvier (Le Genest St. Isle, France) and 60 3-week-old female Wistar rats (*Rattus norvegicus*, HsdCpb:WU) were purchased from Harlan-CPB (Zeist, The Netherlands). We used female animals because we previously found that female rats were more suceptible to changes in plasma cholesterol due to dietary intervention than were male rats[16] and we anticipated that the same might be true for gerbils. The gerbils and rats were housed in groups of three and five animals, respectively, in polycarbonate cages with a wire top and a layer of wood chips as bedding. Room temperature was maintained at 22°C, with 12-hr light:dark cycle (lights on 6:00 AM–6:00 PM). Food intake was measured per cage and expressed per animal per day.

We used coffee oil from the same batch that was used in studies with humans[6] and monkeys.[14] The experiments with humans were carried out in 1992 and the experiments with monkeys, gerbils, and rats in 1991. The animal diets contained either coffee oil or a placebo oil with similar fatty acid composition. The oils were incorporated into semipurified diets (*Table 1*), which were chemically analyzed to verify the composition (*Table 2*). The calculated intake of cafestol is shown in *Table 3*. In various experimental animals the influence of feed components on serum cholesterol is greater in animals fed a cholesterol-rich diet.[18] Therefore, we also used cholesterol-enriched diets in an attempt to enhance the cholesterolemic response to coffee oil. The diets were fed ad libitum in the form of a powder.

On arrival in the laboratory, the gerbils were fed the semipurified diet without cholesterol and coffee oil for a period of 3 weeks. The rats were fed a commercial rodent diet for a period of 3 days, followed by the semipurified diet without cholesterol and coffee oil (*Table 1*) for 2 weeks. After the pre-experimental period, the gerbils and rats were assigned to the various dietary groups so that the groups had similar mean plasma cholesterol concentrations and body weights. The 90 gerbils were divided into 6 groups of 15 gerbils each and transferred to either a cholesterol-free or cholesterol-enriched (0.05%, w/w) diet containing either 0, 0.5%, or 5% (w/w) coffee oil. The 60 rats were divided into 6 groups of 10 animals each and fed a cholesterol-free or cholesterol-enriched diet containing 0, 0.5% or 5% (w/w) coffee oil, just as the gerbils.

Table 1 Composition of the semipurified diets*

Ingredient	g/kg
Casein	151
Coconut oil	25
Corn oil	25
Coffee oil	0/5/50†
Placebo oil	50/45/0†
Cholesterol	0 or 0.05‡
Corn starch	334.275 or 334.225‡
Dextrose	300
Inositol	0.125
Cellulose	50
Taurine	5
$CaCO_3$	12.4
$NaH_2PO_42H_2O$	15.1
$MgCO_3$	1.4
KCl	1
$KHCO_3$	7.7
Mineral mixture§	10
Vitamin mixture§	12

*The energy density of the diet was 16.42 MJ metabolizable energy/kg diet.
†The diets contained 0, 0.5% (8.7 mg cafestol/MJ), or 5% (86.8 mg cafestol/MJ) coffee oil. The coffee oil was added at the expense of the placebo oil; the placebo oil was a mixture of sunflower oil and palm oil (3:2, w/w) and it had a fatty acid composition similar to that of the coffee oil. The calculated fatty acid composition of the placebo oil was 27% saturated fatty acids, 27.7% monounsaturated fatty acids, and 45% polyunsaturated fatty acids (linoleic acid). Fatty acid composition of the palm and sunflower oil were obtained from the USDA Food Tables available on the Internet (www.nal.usda.gov.fnic/cgi-bin).
‡The diets contained 0 or 0.05% (30 mg/MJ) cholesterol and the cholesterol was added at the expense of corn starch.
§Compositions of the mineral and vitamin mixture have been published previously.[17]

Analytical methods

Blood samples were taken after food had been withheld for 16 hr. The gerbils and rats were bled by orbital puncture while under anesthesia with diethyl ether. The animals were sacrificed at the end of the study and the livers removed. Plasma cholesterol concentrations were measured enzymatically[19] with the use of commercial kits (CHOD-PAP kit, Boehringer-Mannheim GmbH,

Table 2 Analyzed composition of the semipurified diets

	Cholesterol-free: % coffee oil			Cholesterol-enriched (0.05%): % coffee oil		
	0	0.5	5	0	0.5	5
	g/kg diet					
Dry matter	929	929	931	938	934	933
Ash	29	31	30	30	30	30
Protein*	138	138	131	131	144	144
Cholesterol	0.038	0.045	0.018	0.580	0.490	0.500
Fat	97	97	97	102	103	100
Fatty acids†						
Saturated	39	40	47	41	42	49
$C_{12}+C_{14}+C_{16}$	31	32	37	33	34	38
Monounsaturated	22	21	13	23	23	13
Polyunsaturated	35	36	37	37	38	38

*Nitrogen content × 6.25 (Kjeldahl factor).
†g/100g fatty acid methyl esters.

Table 3 Calculated intake of coffee oil and cafestol in the present studies with gerbils and rats and in a study with humans*

		% Coffee oil in diet		
		0.5	5	0.5
	Humans	Gerbils		Rats
Body weight	70 kg	58† g	58† g	208† g
Food intake				
g dry matter/d	500	4.15‡	3.96†	11.5‡
g/day/kg body wt	7.1	71.6	68.3	55.3
Energy intake§				
kJ/d	10,000	68.1	65.0	188.8
kJ/d/kg body wt	140	1,176	1,121	908
Coffee oil intake				
g/d	2.000	0.021	0.198	0.058
g/d/kg body weight	0.029	0.358	3.414	0.276
g/MJ	0.200	0.305	3.045	0.305
Cafestol intake‖				
mg/d	57.0	0.6	5.6	1.7
mg/d/kg body weight	0.8	10.2	97.3	7.9
mg/MJ	5.7	8.7	86.8	8.7
Change in cholesterol				
mmol/L	1.27	−0.38/0.24**	1.01/1.87**	0.46/0.28**
%	28	−12/9**	33/70**	27/15**

*A typical subject participating in our published studies.[6]
†Average body weight of the rats and gerbils on the cholesterol-free and cholesterol-enriched diets at the end of the experiments.
‡Average food intake of the rats and gerbils on the cholesterol-free and cholesterol-enriched diets during the experiment.
§The semipurified diets had a calculated energy content of 16.42 MJ/kg diet. See also *Table 1*.
‖The coffee oil (mixture of arabica and robusta oil) contained 28.5 mg cafestol and 34.5 mg kahweol/gram.
**In the absence and presence (right from slash) of 0.05% (30 mg/MJ) dietary cholesterol.

Mannheim, Germany) and an autoanalyzer (COBAS-BIO, Roche, Switzerland). Total liver lipids were extracted[20] and the cholesterol concentration in the liver extract was measured.[21] Aliquots of the diets were placed in a vacuum oven at 70°C or 550°C and the dry matter and the ash content, respectively, were measured. Nitrogen in the diets was measured with the Kjeldahl method.[22] Crude fat was determined by extraction with the Soxhlet method,[22] and fatty acids[23] and cholesterol[24] by gas-liquid chromatography. The diterpene alcohols cafestol and kawheol in the coffee oil were analyzed in ether extracts by capillary gas chromatography, and the purity of peaks was verified by mass spectrometry.[6]

Statistical analysis

Plasma cholesterol concentrations were measured at several time points. Therefore, we used a two-way repeated measures analysis of variance (ANOVA) of cholesterol concentrations with diet and week as factors. The other variables were analyzed with a two-way ANOVA with dietary cholesterol and coffee oil as factors. When the ANOVA test indicated a significant effect of coffee oil and cholesterol in the diet, a pairwise multiple-comparison procedure (*t*-test with the Bonferroni adaptation) was used to test which groups or time points were significantly different.

Results

Gerbils

In four of the six dietary groups, one to three gerbils died during the experiment. This included the two groups not receiving coffee oil. Death was due to Tyzzer's disease as diagnosed by a veterinarian; the remaining gerbils were apparently healthy. The results of the deceased gerbils were not used for further data analysis. There was a trend toward lower feed intake and body weight when increasing proportions of coffee oil were included in the diets (*Table 4*). Plasma cholesterol concentrations in the gerbils fed the diet with 5% coffee oil increased whereas those in the gerbils fed either the 0.5% coffee oil diet or no coffee oil decreased (*Figure 1*). Plasma cholesterol concentrations in the gerbils fed the 5% coffee oil diet were significantly higher throughout the study than in the groups fed no coffee oil or 0.5% coffee oil. There was no difference in plasma cholesterol between the gerbils fed no coffee oil and 0.5% coffee oil. Liver weights were also significantly higher in the gerbils fed the 5% coffee oil diet compared with the gerbils on the diets with either 0.5% coffee oil or no coffee oil. There was a significant correlation between plasma cholesterol and liver weight ($r = 0.55$, $P < 0.001$, $n = 83$, Pearson product-moment correlation). There was no significant interaction between dietary cholesterol and coffee oil with respect to any of the variables measured (*Table 4*).

Rats

The two groups of rats fed the 5% coffee oil diet became sick, food and water intake decreased, and they lost hair. Therefore, these animals were taken off the diet. Food intake and body weight did not differ significantly among the four remaining dietary groups (*Table 5*). Plasma cholesterol concentrations throughout the experimental period were lower than the initial values in all the dietary groups. Repeated measures ANOVA indicated that the rats fed diets containing 0.5% coffee oil had significantly higher plasma cholesterol concentrations throughout the experimental pe-

Table 4 Food intake, body and liver weights, and plasma cholesterol concentrations in gerbils fed semipurified diets containing coffee oil for a period of 10 weeks*

	Cholesterol-free: % coffee oil			Cholesterol enriched (0.05%): % coffee oil		
	0	0.5	5	0	0.5	5
Number of gerbils	13	14	15	12	15	14
Food intake, g/d	4.19 ± 0.36	4.12 ± 0.41	4.07 ± 0.35	4.19 ± 0.25	4.17 ± 0.31	3.85 ± 0.43
Body weight						
Initial	49.4 ± 4.6	49.0 ± 4.3	48.8 ± 3.9	48.8 ± 4.9	48.9 ± 4.1	49.2 ± 4.8
Final, 10 weeks	60.6 ± 3.7	58.3 ± 6.0	57.7 ± 4.3	60.9 ± 7.8	59.6 ± 5.2	57.8 ± 4.3
Plasma cholesterol, mmol/L						
Initial	3.96 ± 0.69	3.81 ± 0.80	3.99 ± 0.76	4.00 ± 0.60	4.08 ± 0.59	3.89 ± 0.72
Final, 10 weeks	3.05 ± 1.04^{b,c}	2.67 ± 0.44^{c}	4.06 ± 0.67^{a,b}	2.67 ± 0.70^{c}	2.92 ± 0.79^{c}	4.54 ± 0.87^{a}
Liver weight, g	1.91 ± 0.17^{b}	1.96 ± 0.32^{b}	2.96 ± 0.32^{a}	1.93 ± 0.36^{b}	2.04 ± 0.29^{b}	3.00 ± 0.29^{a}
Liver weight, %	3.19 ± 0.22^{b}	3.34 ± 0.28^{b}	5.14 ± 0.30^{a}	3.19 ± 0.27^{b}	3.41 ± 0.31^{b}	5.20 ± 0.46^{a}

*Values are means ± SD. Values in a row not sharing a common superscript are signifciantly different ($P < 0.05$). There was no interactive effect of coffee oil and cholesterol.

riod than the groups fed no coffee oil. This effect was more pronounced for the cholesterol-free diets (*Figure 1*) but there was no significant interaction between coffee oil and cholesterol (*Table 5*). Liver weights tended to be higher in rats fed coffee oil, especially in the cholesterol-rich diets (*Table 5*). There was a significant interaction of dietary coffee oil and cholesterol with regard to liver cholesterol. Further, there was a significant correlation between liver weight and plasma cholesterol ($r = 0.38$, $P < 0.02$, $n = 40$) or liver cholesterol ($r = -0.63$, $P < 0.001$, $n = 40$).

Discussion

The objective of the present study was to determine whether the hypercholesterolemic effect of cafestol in coffee oil that is seen in humans[6] could be reproduced in gerbils and rats. We found that diets containing 0.5% coffee oil raised cholesterol by 15 to 27% in rats. In gerbils only diets containing 5% coffee oil were hypercholesterolemic. The proportions of cafestol and coffee oil in the diets with 0.5% coffee oil, if expressed in grams per megajoule of metabolizable energy, were similar to those fed to humans. Gerbils and rats, however, have a considerably higher energy intake per kilogram of body weight than do humans (*Table 3*). As a consequence, the coffee oil intake in the rats and gerbils receiving the 0.5% diets was approximately 10-fold higher and in the gerbils receiving the 5% diets 100-fold higher than that in humans when expressed per kilogram of body weight.

In all our studies with animals[10,14] and humans[6] we used the same batch of coffee oil. The animal studies were done in 1991 and the studies in humans in 1992; thus there was a time span of approximately 1 year between the animal and human studies. The coffee oil was stored at 4°C. Cafestol, the cholesterol-raising factor of the coffee oil, and kawheol are very stable and can withstand temperatures higher than 200°C. Thus, it is unlikely that storage affected the cafestol and kawheol in the coffee oil. Further, the coffee oil contains predominantly saturated and monounsaturated fatty acids and linoleic acid, and almost no trienes (see *Table 1*, composition of placebo oil), and we expected no major changes in these fatty acids under the storage conditions used. Moreover, analysis of other batches of coffee oil at different time points indicated that virtually no changes had occurred during storage. Further, possible changes in the fatty acids of the coffee oil probably would not have

Table 5 Food intake, body and liver weights, and plasma and liver cholesterol concentrations in rats fed semipurified diets containing coffee oil for a period of 8 weeks*

	Cholesterol-free: % coffee oil		Cholesterol-enriched (0.05%): % coffee oil	
	0	0.5	0	0.5
Number of rats	10	10	10	10
Feed intake	10.8 ± 1.01	11.4 ± 0.34	11.5 ± 0.55	11.5 ± 0.56
Body weight				
Initial	105.0 ± 9.5	104.9 ± 6.8	104.6 ± 6.9	104.3 ± 9.0
Final, 8 weeks	208.5 ± 18.7	207.9 ± 14.2	220.5 ± 12.6	211.7 ± 14.8
Plasma cholestserol, mmol/L				
Initial	2.64 ± 0.20	2.66 ± 0.28	2.64 ± 0.23	2.62 ± 0.22
Final, 8 weeks	1.69 ± 0.26^{b}	2.15 ± 0.17^{a}	1.92 ± 0.26^{a,b}	2.20 ± 0.29^{a}
Liver weight, g	6.60 ± 0.84	6.94 ± 0.70	7.01 ± 0.64	7.42 ± 0.67
Liver weight, %	3.16 ± 0.24^{b}	3.33 ± 0.17^{a,b}	3.18 ± 0.18^{b}	3.51 ± 0.67^{a}
Liver cholesterol, μmol/g	7.49 ± 0.53^{b}	7.87 ± 0.90^{a,b}	7.44 ± 0.47^{b}	8.64 ± 0.46^{a}

*Values are means ± SD, $n = 10$. Values in a row not sharing a common superscript are significantly different ($P < 0.05$). There was a significant interactive effect of coffee oil and cholesterol on liver cholesterol concentrations.

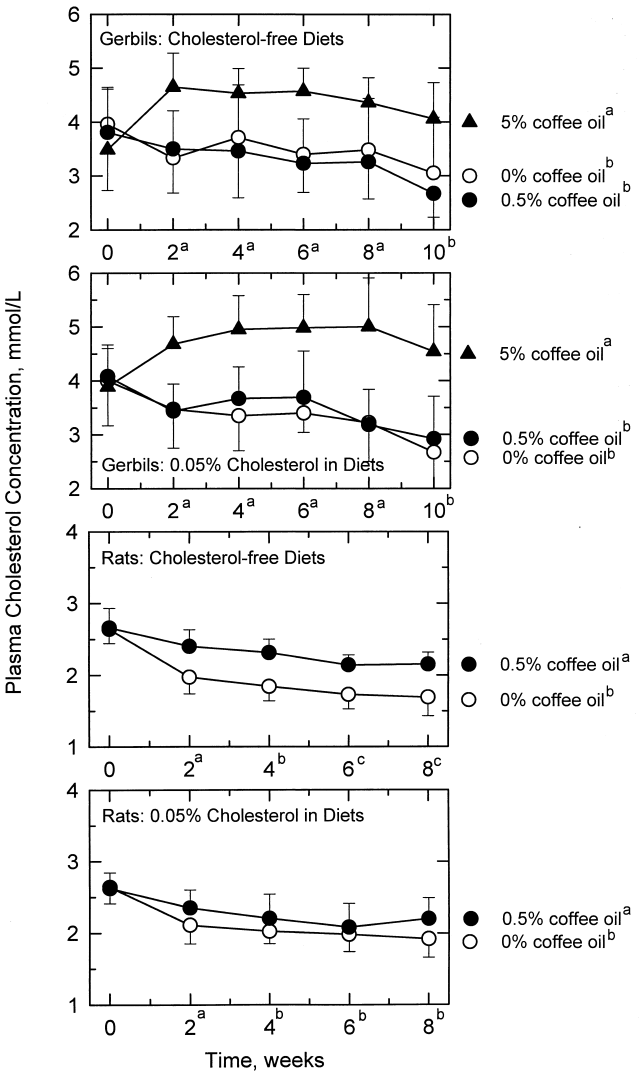

Figure 1 Plasma cholesterol concentrations (means ± SD, numbers of animals for the various dietary groups are given in *Tables 4* and *5*) in gerbils and rats fed semipurified diets containing containing various proportions of coffee oil and cholesterol. We used a two-way repeated measures analysis of variance (ANOVA) of cholesterol concentrations with diet and time as factors. When the ANOVA test indicated a significant diet or time effect, a pairwise multiple-comparison procedure (*t*-test with the Bonferroni adaptation) was used to test which time points or diets were significantly different from each other. Dietary groups and time points that do not share a common superscript are significantly different ($P < 0.05$).

affected the hypercholesterolemic effect of the coffee oil because this effect is caused by the cafestol rather than the fatty acids.

In earlier studies with rats unfiltered coffee did not affect cholesterol. We think this is due to the lower doses of cafestol fed to animals in these studies. We calculated that in the studies of Beynen et al.[10] and Høstmark et al.[13] the rats were fed only 1.1 and 2.7 mg cafestol/MJ, respectively (*Table 6*). In the present study the intake of cafestol was 8.7 mg/MJ. The calculated cafestol intake in studies of Al-Kanhal et al.[12] was 4.5 to 4.8 mg/MJ and this caused a significant increase in plasma cholesterol of 0.59 mmol/L (34%) in animals fed a cholesterol-free diet and of 0.34 mmol/L (18%) in animals fed a cholesterol-rich diet (*Table 6*). These effects are comparable to the increases found in our study [i.e., 0.46 mmol/L (27%) and 0.28 mmol/L (15%)]. In the studies of Rakicioglu et al.[25] the cafestol intake was 15.2 mg/MJ, which raised plasma cholesterol levels by 30% and 57% in rats fed a cholesterol-free or cholesterol-rich diet, respectively.

Mensink et al.[11] reported that feeding freeze-dried boiled coffee to gerbils did not raise plasma cholesterol. We calculated that they fed 4.3 mg cafestol/MJ (*Table 6*). In our study the gerbils fed the 0.5% coffee oil diet had an intake of 8.7 mg cafestol/MJ (*Table 3*), which is higher than that in the study of Mensink et al.,[11] but which still did not affect plasma cholesterol. Higher plasma cholesterol levels in gerbils was found only when 5% coffee oil was added to the diet. It would thus appear that gerbils are less sensitive to coffee oil than rats. Gerbils also seem to be more resistant to toxic effects of coffee oil as seen in the rats fed the 5% coffee oil.

In a previous study[14] we fed Cebus and Rhesus monkeys a diet containing 7.7 mg cafestol/MJ, an amount comparable to that fed to humans[6] (5.7 mg cafestol/MJ). This dose of cafestol did not raise plasma cholesterol. Thus, it appears that these species of monkeys, like gerbils, are less sensitive to cafestol than humans.

Studies in hamsters appear to be conflicting (*Table 6*). Beynen et al.[10] found no effect of boiled coffee on plasma cholesterol when a dose of 5.3 mg cafestol/MJ was fed. On the other hand, Sanders and Sandadura[8] and Ratnayake et al.[9] reported a significant increase in plasma cholesterol after feeding hamsters a dose of 1.5 mg/MJ (in the form of boiled coffee) and 2.2 mg cafestol/MJ (in the form of coffee oil), respectively. Higher doses of cafestol were fed in the studies of Mensink et al.[11] (4.3 mg cafestol/MJ) and Ratnayake et al.[9] (6.8–8.9 mg cafestol/MJ), but these amounts still did not affect plasma cholesterol. Thus, differences in doses of dietary cafestol cannot explain these different results.

A major question is whether the observed hypercholesterolemic effect of coffee oil in rats and gerbils is caused by a specific action of coffee oil or is secondary to toxic effects of this oil. Coffee oil at a level of 5% in the diet was found to be toxic to rats. Furthermore, the feeding of coffee oil produced increased liver weights in the gerbils and rats, which could reflect a hepatoxic action. The intake of boiled coffee or coffee oil raised the activity of serum alanine aminotransferase and aspartate aminotransferase in humans, indicating disturbed liver function.[6] Urgert and Katan[7] have suggested, however, that a perturbation of liver cell function is unlikely to explain the effects of coffee diterpenes on blood lipids in humans, because both cafestol and kawheol raise aminotransferases whereas kawheol has little effect on blood lipids. Therefore, we would suggest that the hypercholesterolemic effect of coffee oil in rats is caused by a specific action of coffee oil and cafestol rather than by a toxic effect of the oil.

In the gerbils and the rats fed no coffee oil and 0.5% coffee oil, the plasma cholesterol concentrations throughout the experimental period were lower than the initial values. This decrease in plasma cholesterol may be an age or time

Table 6 Effects of cafestol consumption on plasma cholesterol concentrations in humans, rats, gerbils, and hamsters

Reference	Cholesterol in diet %, w/w	Cholesterol in diet mg/MJ	Cafestol in diet (mg/MJ)	Source of cafestol	Number per group	Plasma cholesterol (mmol/L*) Control	Plasma cholesterol (mmol/L*) Cafestol	Increase mmol/L	Increase %
Humans									
Weusten-van der Wouw et al.[6]	—	28	5.7	Coffee oil	15	4.48	5.70[b]	1.27	28
Rats									
Høstmark et al.[13]	0	0	2.7[†]	Boiled coffee	12	2.47 ± 0.31	2.34 ± 0.35	−0.13	−5
Al-Kanhal et al.[12]	0	0	4.8[†,‡]	Boiled coffee	8	1.75 ± 0.18	2.34 ± 0.738[b]	0.59	34
Al-Kanhal et al.[12]	1	625	4.5[†,‡]	Boiled coffee	8	1.90 ± 0.27	2.24 ± 0.29[b]	0.34	18
Beynen et al.[10]	0	0	1.1	Boiled coffee	13	2.06 ± 0.28	2.43 ± 0.24	0.37	18
Beynen et al.[10]	1	500	1.1	Boiled coffee	13	7.72 ± 3.60	6.56 ± 1.80	−1.16	−15
Rakicioglu et al.[25]	0	0	15.2[‡,§]	Coffee grounds	10	1.27 ± 0.31	1.68 ± 0.64	0.38	30
Rakicioglu et al.[25]	1.2	750	15.2[‡,§]	Coffee grounds	10	1.82 ± 0.63	2.85 ± 0.72[a]	1.03	57
Gerbils									
Mensink et al.[11]	0.5	305	4.3[†,‖]	Boiled coffee[4]	20	4.33	4.29	−0.04	−1
Hamsters									
Sanders and Sandadura[8]	0.1	50	1.5[†]	Boiled coffee	18–20	4.62 ± 0.56	5.40 ± 1.11[b]	0.78	17
Mensink et al.[11]	0.5	294	4.3[†,§]	Boiled coffee[4]	6	6.28	5.67	−0.61	−10
Ratnayake et al.[9]	0.03	19	2.2[**]	Coffee oil	11	2.62	2.98[a]	0.36	14
	0.03	19	1.5[**]	NSM of coffee oil	11	2.62	2.78	0.16	6
	0.03	19	1.7[**]	Diterpenes	11	2.62	2.91	0.29	11
	0.1	50	8.9[**]	Coffee oil	11	3.40	3.28	−0.12	−4
	0.1	50	6.2[**]	NSM of coffee oil	11	3.40	3.52	0.12	4
	0.1	50	6.8[**]	Diterpenes	11	3.40	3.43	0.03	1
Beynen et al.[10]	0	0	5.3	Boiled coffee	13	5.04 ± 1.23	4.79 ± 0.76	−0.25	−5
	0.06	35	5.3	Boiled coffee	13	6.11 ± 0.61	6.19 ± 1.23	0.08	1

*Values are means ± SD. [a]$P < 0.05$; [b]$P < 0.01$. In the studies of Beynen et al,[10] female rats were used, whereas in all the other studies with animals, males were used.
[†]Calculations are based on the assumption that 150 mL of boiled coffee (60 g ground coffee added to 1 L of boiling water) contains 6.2 mg cafestol.[7]
[‡]Calculations are based on the assumption that a commercial diet (5% fat) has an energy density of 16 MJ/kg.[9]
[§]Calculations are based in the assumption that coffee grounds contain 486 mg cafestol/100 g.[26]
[‖]The boiled coffee was freeze-dried and added to the diets.
[**]The calculations are based on an energy intake of 0.14 MJ/day.[10]
NSM–nonsaponifiable matter of the coffee oil.

effect and similar results in young, growing rats fed either a cholesterol-free or cholesterol-supplemented diet have been reported in other studies.[16,27] Nevertheless, the cholesterol levels in the rats fed the 0.5% coffee oil decreased less than did those in the rats fed the control diet, and throughout the study, the rats fed coffee oil had higher plasma cholesterol levels than did the control groups. In the gerbils fed the 5% coffee oil diet, the cholesterol levels increased and the cholesterolemic effect of the coffee oil probably overruled the decrease in cholesterol levels due to age or time. In adult humans, coffee oil not only resulted in higher cholesterol values compared with the control group, but also resulted in higher cholesterol levels compared with the initial values.[6] The use of adult rats and gerbils rather than the use of young and growing animals may also give a similar pattern of cholesterolemic response as seen in humans.

In conclusion, the results of our studies indicate that feeding cafestol induces higher plasma cholesterol levels in gerbils and rats. In the rat, the proportions of cafestol in the diet that are needed to induce changes in plasma cholesterol levels are similar to those in humans. Gerbils, however, are less sensitive than rats. In gerbils, a 10-fold higher proportion of cafestol must be added to the diet to induce a cholesterolemic effect of cafestol that is comparable to that found in humans and rats. Further, the available literature suggests that only humans and rats respond consistently to cafestol. Results in hamsters are conflicting and data on gerbils and Cebus and Rhesus monkeys suggest that these species are less sensitive than are humans. Thus, the rat appears a useful animal model to study the hypercholesterolemic effects of cafestol, as seen in humans.

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